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Using micro technology in process screening for improved ω-transaminases

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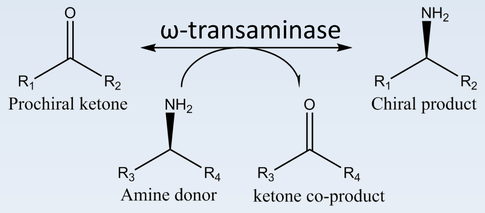
Introduction:

Today, optically pure chiral amines are particularly important intermediates in a broad range of pharmaceuticals[1]. Biocatalysts provide the possibility of having high selectivity combined with mild reaction conditions and simplified downstream processing [2, 3]. One biocatalyst of interest for this synthesis is ω-transaminase, which provides a unique opportunity towards the asymmetric production of optical pure chiral amines, with >99 % e.e., compared to conventional methods (scheme 1).



Scheme 1: The asymmetric synthesis of optically pure chiral amines from an amine donor and matching ketone.

A big hindrance to the industrial implementation of ω-transaminase is the unfavorable equilibrium[3]. This can partly be circumvented by smart process engineering, but it can only be expected to handle part of the problem. It is therefore highly desired to implement process considerations at an early stage in the overall process development, including integration with protein engineering. A recent review by Bornscheuer and co-workers.[4] describes the impressive contribution that protein engineering can make in process implementation. A key requirement is the further development of effective analytical tools that will be able to handle the "smarter" libraries that today are smaller (10^3 samples), but with a higher chance of finding the right mutant, compared to the past (10^5 samples). In general it is now possible and of great importance to rethink the disposition of work between protein and process engineers. Today it is clear that the two fields can complement each others weaknesses. The strategies of process engineering with regard to reaction optimisation are illustrated in **Figure 1** and **2**. In **Figure 2** the strategies for direct protein engineering are also shown.

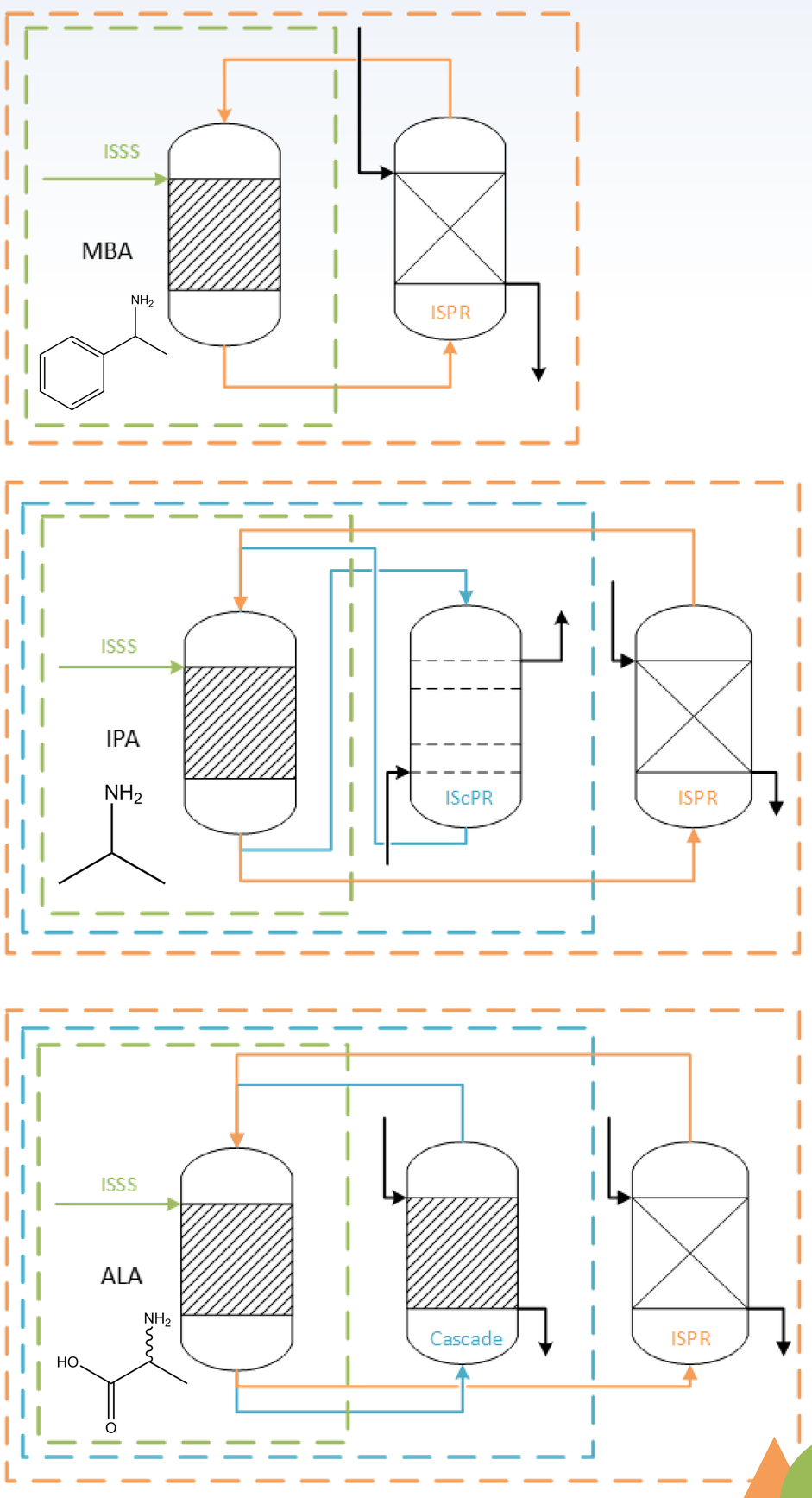


Figure 1— Flowsheets that can be used as a basis to optimise reactor performance, ISSS—In-situ substrate supply, ISPR—in-situ product removal, IScPR—in-situ co-product removal, Cascade– cascade enzymes

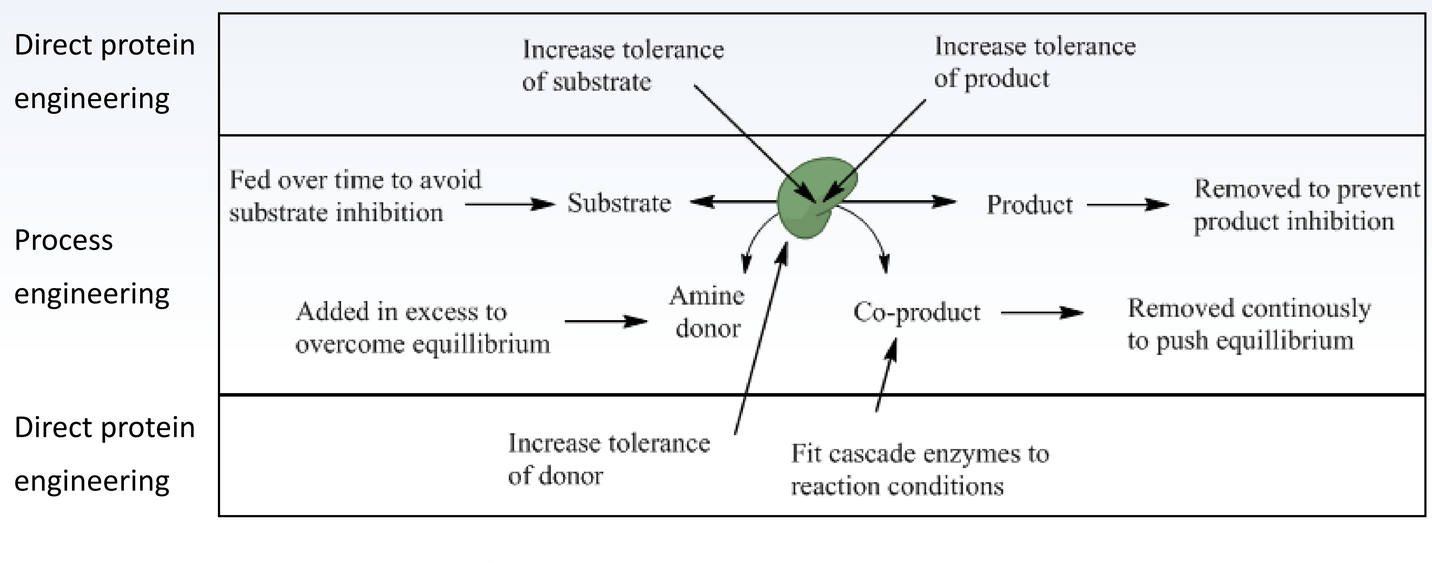


Figure 2— The different strategies of process engineering and direct protein engineering.

In **Figure 1** the schemes has been made according to donor (MBA, IPA or ALA), and displays operations by color coding. A complete overview of the strategies can be seen in **Table 1**. Direct protein engineering affects the rate and the selectivity of the protein, whereas in-direct affects the stability and optimal environment.

Table 1— Overview of the different strategies

	Process engineering	Direct protein engineering	In-direct protein engineering	Driver
Substrate	Feed substrate	Increase tolerance	Positive solvent effect on activity	• Minimum rate([S]) • Price of enzymes
Amine donor	Add in excess		Increase tolerance and stability	• Yield
Co-product	In-situ co-product removal	Fit cascade enzymes to reaction conditions	Increase tolerance and stability and temperature stability.	• Yield
Product	In-situ product removal	Increase tolerance	Solvent and pH tolerance	• Product concentration

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